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OÖCYTE DEVELOPMENT IN *DIPLOPTERA PUNCTATA* (ESCHSCHOLTZ) (BLATTARIA)

L. M. ROTH* and BARBARA STAY†

Pioneering Research Laboratories,
Quartermaster Research and Engineering Center,
Natick, Massachusetts

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Abstract—Mating stimuli activate the corpora allata in female *Diploptera* principally during or after insertion of the spermatophore into the bursa copulatrix and about the time the male releases the female. Copulation, prior to spermatophore transfer, is a comparatively poor stimulus for oöcyte maturation. Inhibition of the corpora allata by uterine eggs during most of the pregnancy period is under nervous control. It is suggested that receptors (mechano- or pressure-receptors) which receive the stimulus from the stretched uterus become adapted during the late stages of pregnancy, or perhaps adaptation occurs in the central nervous system. Consequently, a few days before parturition, inhibition of the corpora allata ceases or is reduced, and yolk deposition occurs in the oöcytes. Severing the nerve cord prior to parturition, and before the oöcytes contain yolk, results in premature development of the oöcytes. Transecting the nerve cord does not prevent oöcyte maturation which is evidence against the hypothesis that genital stimulation, from movement of the oötheca, initiates the activity of the corpora allata.

INTRODUCTION

AMONG the species of cockroaches that incubate their eggs in a brood sac or uterus, *Diploptera punctata* is the most highly evolved from the standpoint of viviparity (ROTH and WILLIS, 1958). It is the only known cockroach in which the developing embryos derive nourishment, other than yolk or water, from the female. The small size and number of eggs make it possible for the female, during oviposition, to transfer the oötheca into her brood sac without exposing the majority of eggs outside her body (ROTH and WILLIS, 1955). Oviposition by 'ovoviviparous' cockroaches is similar to that of oviparous forms, except that in the latter, the oötheca is deposited outside the body of the female on the substrate (ROTH and WILLIS, 1954). Morphologically, the reproductive system of *Diploptera* is comparable to that of oviparous cockroaches, although some of the structures in the former have become modified with its altered physiology (HAGAN, 1941). Like other species of Blattaria that incubate their eggs internally, *Diploptera* has two birth products, the egg and larva (ROTH and WILLIS, 1958). When we speak of ovulation or oviposition we refer to the eggs being released from the ovaries, oriented in two rows and covered by the oötheca. By parturition we mean the birth of larvae following a period of pregnancy or gestation in the uterus.

* Present address: Central Research Laboratories, United Fruit Company, Norwood, Massachusetts.

† Present address: Department of Biology, University of Pennsylvania, Philadelphia, Pa.

The brain of virgin females of *D. punctata* inhibits the corpora allata, and, consequently, maturation of the oöcytes is delayed or prevented. Mating stimuli from the genital region, transmitted via the nerve cord to the brain, activate the corpora allata, and the oöcytes mature. During pregnancy, growth of the oöcytes is again inhibited as a result of a nervous factor, and possibly a chemical substance, originating from the developing uterine eggs, acting on the brain to inhibit the activity of the corpora allata. Stimuli originating in the genital region prior to and during parturition are believed to activate the corpora allata (ENGELMANN, 1959, 1960).

In this paper we describe our experiments on oöcyte development in *Diploptera*. In many respects our data are in agreement with ENGELMANN's, but our interpretation of some of the mechanisms of oöcyte inhibition or stimulation differs.

MATERIALS AND METHODS

The insects were fed dog chow checkers and kept at 24–25°C and 50–70 per cent relative humidity. ENGELMANN (1959) has shown that yolk deposition and growth of the oöcytes are correlated with and dependent upon the activity of the corpora allata. We have used oöcyte development as an indicator of endocrine activity. Measurements were made of oöcytes and uterine eggs that were dissected from ovaries or oöthecae in Ringer's solution. The basal oöcytes in an ovary are fairly uniform in size and only one oöcyte per female was measured. In establishing the normal sizes of oöcytes at different stages in the ovarian cycle a large number of females was dissected; the differences in size of the oöcytes among these individuals give a more reliable picture of the variation than if several oöcytes in each ovary are measured in a relatively small sample of females. Various operations were carried out on insects kept under carbon dioxide anaesthesia.

RESULTS AND DISCUSSION

The ovarian cycle

The sizes of oöcytes of virgins and mated females, and the growth of the oöcytes following parturition are given in Table 1. The ovarian cycle from emergence to the second oviposition is shown in Fig. 1. ENGELMANN (1959) found that the oöcytes grow from about 0·55 mm to approximately 1·5 mm and then oviposition occurs. Our measurements of the oöcytes were larger than the values recorded by ENGELMANN. This discrepancy is probably due to his fixing the eggs prior to measuring them, whereas we dissected and measured eggs in Ringer's solution. During the first 19 days the oöcytes in virgin females varied from 0·54 to 0·87 mm (Table 1); some of the maximum values may represent females in which more than the usual amount of development occurred despite the absence of mating. Based on mean values the oöcytes of virgin females vary from $0{\cdot}62 \pm 0{\cdot}01$ mm to $0{\cdot}72 \pm 0{\cdot}02$ mm. The oöcytes of some virgin females, for reasons unknown, begin to develop, and ovulation may eventually occur. These are plotted separately in Fig. 1 and are not combined with the values of undeveloped oöcytes. The first virgin females we found that had well-developed oöcytes were 20 and 37 days old (Fig. 1), and the first female examined that had an oötheca was 30 days old. Of 394 virgin females examined between the ages of 11 and 172 days (the data beyond 125 days are not plotted in Fig. 1), only 36 (9 per cent) had oviposited and 15 had oöcytes

TABLE 1—OÖCYTE DEVELOPMENT IN *Diploptera punctata*

Age of females (days)	Length of basal oöcytes (mm)									
	Virgins*			Mated†			Following parturition‡			
	Min.	Max.	Mean ± S.E.§	Min.	Max.	Mean ± S.E.	Days	Min.	Max.	Mean ± S.E.
0	0·54	0·67	0·62 ± 0·01	0·54	0·67	0·62 ± 0·01	0	0·71	1·18	1·04 ± 0·04
1	0·64	0·72	0·68 ± 0·01	0·62	0·71	0·67 ± 0·01	1	1·02	1·34	1·18 ± 0·05
2	0·64	0·72	0·69 ± 0·01	0·67	0·77	0·72 ± 0·03	2	1·14	1·36	1·26 ± 0·04
3	0·64	0·77	0·70 ± 0·02	0·69	0·86	0·78 ± 0·02	3	1·18	1·48	1·42 ± 0·08
4	0·67	0·76	0·70 ± 0·01	0·72	1·02	0·90 ± 0·03	4	1·01	1·76	1·48 ± 0·13
5	0·69	0·87	0·72 ± 0·02	1·04	1·34	1·22 ± 0·03	5	1·41	1·76	1·59 ± 0·08
6	0·64	0·79	0·70 ± 0·01	1·11	1·55	1·31 ± 0·04	6	1·71	1·86	1·80 ± 0·03
7	0·59	0·79	0·68 ± 0·02	1·18	1·63	1·46 ± 0·04	7		Oviposition	
8	0·64	0·81	0·69 ± 0·01	1·44	1·80	1·64 ± 0·03				
9	0·67	0·82	0·71 ± 0·01	1·71	1·88	1·78 ± 0·02				
10	0·64	0·84	0·71 ± 0·02		Oviposition					
11	0·64	0·72	0·69 ± 0·01							
12	0·62	0·72	0·68 ± 0·01							
13	0·62	0·76	0·69 ± 0·01							
14	0·59	0·79	0·69 ± 0·02							
15	0·67	0·79	0·70 ± 0·01							
16	0·62	0·74	0·67 ± 0·01							
17	0·64	0·82	0·69 ± 0·01							
18	0·64	0·79	0·69 ± 0·01							
19	0·66	0·73	0·69 ± 0·002							

* Ten females were used for determining each mean value.
† Females were mated at or shortly after emergence; 10 females were used for determining each mean value.
‡ Ten females were used for determining the mean value at 0 day; 5 females were used for obtaining each of the other mean values.
§ In this and the following tables Min. = minimum, Max. = maximum, and S.E. = standard error.

that were definitely developed and larger than the maximum values for virgin females shown in Table 1. ENGELMANN found 1 virgin female which ovulated in 37 days, 8 others in 60 days, and 13 in 2–5 months. After mating, which normally occurs shortly after the female emerges and is still white and teneral (ROTH and WILLIS, 1955), the oöcytes mature rapidly and oviposition occurs 10–15 days later (Table 1). Mature oöcytes may be as large as 1·88 mm but average 1·78 ± 0·02 mm. During gestation, the basal oöcytes increase from about 0·40 mm (at ovulation) to 1·04 ± 0·04 mm at parturition, and ovulation occurs again in about 7 days after birth of young. In our series of females, parturition occurred 99–110 days (103 ± 0·8; $N = 12$) after oviposition. Except for differences in the size of the oöcytes and the time interval of pregnancy (probably due to a difference in temperature) our results are similar to ENGELMANN's.

Starved mated *Diploptera* mature their oöcytes in about the normal period of time (ENGELMANN, 1960). We starved 10 mated females without water, and they all oviposited in an average of $12 \cdot 4 \pm 0 \cdot 5$ days. Twenty mated females starved with

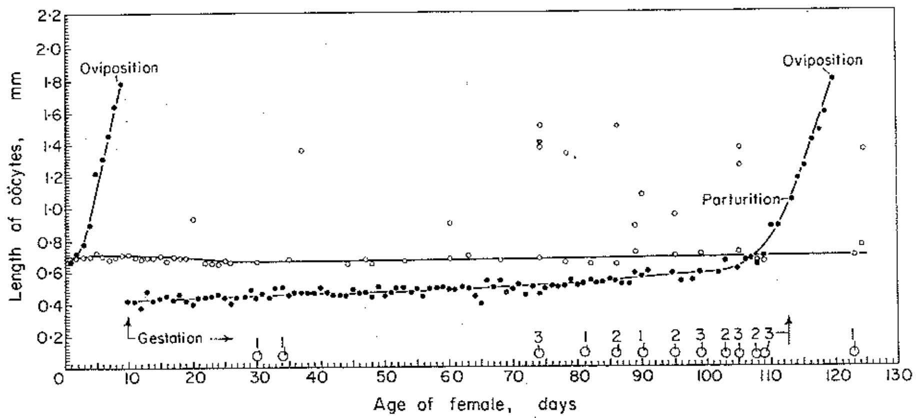

FIG. 1. Ovarian cycle (first and second ovipositions) in *Diploptera punctata.* The data in Table 1 were used to plot the curves for the virgin females (○) up to the nineteenth day, and the first preoviposition and post parturition periods of mated females (●); the measurements of undeveloped oöcytes of virgins, beyond the nineteenth day, are means of four to twelve measurements (total of 224 females). Each measurement of the oöcytes that contained yolk and were larger than 0·87 mm, in virgin females, represents 1 female. The values for mated females during the gestation period represent individual females or are means of two to six measurements (total of 125 females). The numerals attached to circles along the abscissa represent the number of virgin females found with oöthecae in the uterus on the day indicated.

water oviposited on an average of $11 \cdot 6 \pm 0 \cdot 1$ days. Mated, fed, and watered females oviposited in $12 \cdot 4 \pm 0 \cdot 2$ days ($N = 35$). Thus, neither food nor water is necessary for stimulation of oöcyte development in *Diploptera.*

Effect of implanting corpora allata into allatectomized females

Allatectomized mated females do not produce oöthecae. Four mated females were allatectomized at 1 day of age. After 5 and 6 months these females were still carrying spermatophores and had not produced oöthecae. Two pairs of corpora allata from mated females were implanted into these females, 5 and 6 months after allatectomy. Two females did not produce oöthecae, but their spermatophores were loosened and dropped, and their ovaries contained developed (1·51 mm) and degenerating eggs when examined at death of the animals 2–3 months after the implants. Two females produced oöthecae 9–12 days after the implants and several oöthecae subsequently. One of these females produced five oöthecae in a period of 6 months and then no more until her death at 16 months of age. The other female produced three oöthecae in 6 months and no more until death at 12 months of age. Although the females were mated before allatectomy the embryos did not develop in the oöthecae which were produced under influence of implanted corpora allata after 5 and 6 months of allatectomy. Possibly the corpora allata are also necessary for the maintenance of sperm in the spermathecae. A mated female, allatectomized at 1 day of age, received immediately an implant of three corpora allata. She oviposited at 9 days of age and the young were born after about 4 months. The eggs in the next oötheca, which she produced 4 months later, also developed, but the female died before the young were born.

Our observations confirm ENGELMANN's (1959, 1960) findings that the corpora allata are necessary for the release of the spermatophore and for the maturation of eggs in *D. punctata*.

Effect of implanting corpora allata into virgin females

The effect of mating can be mimicked by implanting corpora allata from mated females into virgins. Four virgin females received implants of corpora allata—less than 1 day after metamorphosing and all oviposited between 9 and 15 days following implantation. Subsequently three of the virgin females produced from two to five oöthecae on the average of one every 5 weeks. ENGELMANN (1960) studied the effect of implanting corpora allata into pregnant *Leucophaea maderae* (F.) and found that isolated active corpora allata could stimulate as many as five egg maturations within 8 months. Our experiments with implantation of active corpora allata into virgin females of *D. punctata* show that, also in this species, isolated corpora allata are capable of stimulating the maturation of several batches of eggs in a much shorter time than would occur normally in a mated female.

Mating and oöcyte development

A series of experiments was performed to determine the importance of different parts of the copulatory act in stimulating oöcyte development. The effect of interrupting mating prior to transfer of the spermatophore was investigated. Two methods of separating the mating pair were used. In the first, the male and female were manually separated by simply pulling them apart. When this was done and observed under the microscope, it was seen that the genital region of the female, which was clasped by the male's phallomere, was pulled out and stretched before the male released his hold. In the second method the pair was anaesthetized with carbon dioxide, and the male slowly released his hold so that no undue pressure was applied to the female's genital region.

The effect of interrupting mating on subsequent development of the oöcytes is shown in Table 2. Of the 39 females that were separated from males while *in copula*, but prior to transfer of the spermatophore, 31 (80 per cent) showed no development of oöcytes other than what might be found in virgins; 1 female that was joined to a male for 6 hr also failed to develop her oöcytes. Of the 8 positive cases 2 oviposited and 6 had oöcytes that had developed but were smaller than one finds in mated females of the same age. In contrast, oöcytes in females that have spermatophores inserted in the bursa during mating almost invariably develop (see below).

STAY and ROTH (1958) found that two factors influence the duration of copulation in *Diploptera*—age and time since previous copulation. Males younger than 20 days take longer to complete copulation than older individuals; males 20–35 days of age complete copulation in an average of 30–33 min. The duration may be greatly prolonged if males mate a second time shortly after the first. It appears from Table 2 that it is not necessarily the length of time a pair is *in copula* that is

TABLE 2—EFFECT OF INTERRUPTING MATING OF *Diploptera punctata*, PRIOR TO SPERMATOPHORE TRANSFER, ON SUBSEQUENT DEVELOPMENT OF THE OÖCYTES

Time spent *in copula** (min)	Age of females when dissected or oviposited (days)	Oöcytes Mean ± S.E. (mm)	*N*†
Separated by anaesthesia			
10–30	8–14	0·72 ± 0·02	10
5	14	Oviposited	1
Separated manually			
2–39	6–12	0·72 ± 0·02	20
360‡	10	0·67	1
27–35	11	1·14 ± 0·41§	6
	11	Oviposited	1

* Females mated on the day of emergence. The males were 21 days old and at this age they take about 30 min to mate (STAY and ROTH, 1958).

† In this and the following tables *N* = number of insects.

‡ The male used with this female had recently mated and though the pair was joined for 6 hr no spermatophore had been transferred when they were separated.

§ When separated from the male 1 female had a small amount of male accessory gland secretion on her ovipositor; this female had the largest oöcytes (1·56 mm) of the 6 individuals in this group.

of prime importance in stimulating the development of the oöcytes, but some other factor in the mating act—perhaps the insertion of the spermatophore or its presence in the bursa.

The effect of removing the spermatophore from females at various times after mating is shown in Table 3. All of the females oviposited and the results indicate that once the spermatophore has been inserted into the bursa, the oöcytes develop and ovulation occurs in the normal period of time. This was true even when the spermatophore was removed after the mating pair had just separated. Apparently it took about 5 hr for sperm to migrate into the spermathecae since uterine eggs failed to develop when the spermatophore was removed earlier than this. It seems that stimulation resulting from mating becomes effective during the act of spermatophore transfer, or at the time the male releases the female after the spermatophore has been inserted into the bursa copulatrix.

To determine the relative importance of spermatophore transfer and normal release of the female by the male after completion of copulation, females were mated to males lacking accessory glands so that no spermatophore was transferred. For controls, females were mated to males lacking testes. The results are shown in Table 4. Stimulation of the corpora allata in females mated to males lacking accessory glands is not as effective as that resulting when females are mated to males lacking testes. Only 6 of the 26 females mated to males lacking accessory glands oviposited by the fourteenth day; 11 dissected 10–15 days after mating had oöcytes that were comparable in size to those of 6-day-old mated females, and 9 failed to develop their oöcytes. In contrast, of the 41 females mated to males lacking testes,

TABLE 3—EFFECT OF REMOVING SPERMATOPHORES FROM *Diploptera punctata*, AT DIFFERENT TIMES AFTER MATING, ON SUBSEQUENT DEVELOPMENT OF THE OÖCYTES

Time after mating spermatophore was removed from female	Time to oviposit (days)	Development of uterine eggs (+ or −)	*N*
0·5 min or less*	10–12	−	3
1 min or less	10–14	−	5
2–15 min	10–13	−	9
1–4·3 hr	12–15	−	8
5–5·7 hr	11–15	+†	4
7 hr	12	+	2
7 hr	12	−	1
19–28 hr	12–15	+	12

* In recently mated females the spermatophore is readily removed because the accessory gland secretion of the male has not yet hardened around the inserted spermatophore. The older the spermatophore the more difficult it is to remove, until several days before ovulation when a secretion from the anterior female accessory sex glands (ENGELMANN, 1960) softens the hardened material facilitating extrusion or removal of the spermatophore.

† Only 1 of the 4 females was kept and her spermatophore had been removed 5 hr and 14 min after mating; the uterine eggs of this female developed.

TABLE 4—EFFECT OF MATING MALES LACKING ACCESSORY GLANDS, OR TESTES, ON SUBSEQUENT OÖCYTE DEVELOPMENT IN *Diploptera punctata*

Condition of males	Age of females when dissected or oviposited (days)	Oöcytes Mean ± S.E. (mm)	*N*
Lacking accessory glands	10–13	0·71 ± 0·02	9
	10–15	1·27 ± 0·05	11
	14	Oviposited	6
Lacking testes*	18	0·77	1
	9–12	1·68 ± 0·04	5
	10–14	Oviposited	35

* Testes were removed from adults and larvae. In the former, the spermatophores contained sperm since sperm were already present in the seminal vesicles of newly emerged males; the uterine eggs of females mated to these males developed normally. No sperm were present in the spermatophores of males resulting from nymphs that had their testes removed; uterine eggs from females mated to these males did not develop.

35 oviposited, 5 had oöcytes about the size of those in mated females 8 days old, and only 1 failed to develop its oöcytes.

Insertion of the spermatophore by the male enhances stimulation of the corpora allata. However, mating alone, provided it is allowed to go to 'completion', i.e. until the male voluntarily releases the female (even though no spermatophore is transferred), may result in sufficient stimulation for some females to mature their oöcytes. It was often noted that just prior to the male's release of the female he would move his abdomen from side to side which may be an added stimulus in addition to spermatophore transfer.

TABLE 5—EFFECT OF CUTTING THE VENTRAL NERVE CORD ON SUBSEQUENT DEVELOPMENT OF THE OÖCYTES IN *Diploptera punctata*

Nerve cord severed	Age of females when dissected or oviposited (days)	Oöcytes Mean ± S.E. (mm)	*N*
Prior to mating*	15–16	0·68 ± 0·01	7 (–)§
After mating†			
3–180 min	8–19	0·67 ± 0·01	7 (–)
1 day	20	0·69 ± 0·04	9 (–)
2 days	9	0·66	1 (–)
3 days	10–24	0·76 ± 0·02	3 (–)
3 days	24	1·26	1 (⊥)‖
4 days	9	0·81 ± 0·04	3 (–)
6 days	14–15	1·49 ± 0·06	2 (⊥)
6 days	13	Oviposited	5 (+)¶
7 days	11	1·59 ± 0·04	2 (⊥)
7 days	20	1·27 ± 0·26	2 (–)
7 days	12–13	Oviposited	2 (+)
8 days	13	1·48 ± 0·03	2 (–)
8 days	19	1·39	1 (–)
8 days	12	Oviposited	3 (+)
Controls (unoperated)			
Unmated‡	15–22	0·67 ± 0·02	6
	22	0·89	1

* Nerve cord severed and female mated when less than 1 day old.
† Females mated shortly after emergence when still white and teneral.
‡ See Table 1 for lengths of oöcytes of mated females at various days after copulation.
§ (–) = Oöcytes did not develop after the nerve cord was severed.
‖ (⊥) = Oöcytes developed after nerve cord severance but not to the same extent as in unoperated mated females.
¶ (+) = Oöcytes developed normally after nerve cord severance.

The question may be raised whether the nervous stimulus during mating is a triggering action at the time of spermatophore transfer and of release of the female by the male, or whether further stimulation from the genital region, after mating is completed, is needed for maturation of the oöcytes. To determine this, the nerve cords of females were severed before and at various times after mating.

The results are shown in Table 5. As ENGELMANN (1959) found, severing the nerve cord prior to mating prevents oöcyte development. However, severing the nerve cord even up to the eighth day after mating resulted, in some cases, in a cessation of oöcyte development or only a slight increase in oöcyte lengths (cf. Tables 5 and 1). When the nerve cords were severed 6–8 days after mating, the eggs continued to grow in 10 of 19 females and matured in the normal period of time. ENGELMANN (1959) found that the activity of the corpora allata increases during the first preoviposition period until a peak of activity is reached 4 days after mating. The gland then maintains a high activity for about 4 more days. It appears from our nerve cord severance experiments that the increase in activity of the corpora allata is dependent upon an intact nerve cord and that stimulation from the genital region for several days after mating is necessary for oöcyte maturation. In *Leucophaea*, mating is an effective stimulus only if the nerve cord remains intact for at least 2 days after copulation, and ENGELMANN (1960) suggests that this indicates that the effect of mating is not caused solely by a nervous mechanism. It is possible that the presence of a spermatophore in the bursa in *Diploptera* results in nervous stimuli during most of the preoviposition period. Even though removal of the spermatophore just after mating is completed does not prevent oöcyte development it should be pointed out that some accessory gland material from the male is left in the genital region of the female after this operation, and also that the female had already been stimulated by insertion of the spermatophore. However, females may develop their oöcytes when mated even though no spermatophore is transferred (admittedly this may not be as effective as a normal mating) which indicates that the stimulus is essentially a mechanical one. Spermatophore transfer may be a more effective mechanical stimulus than the clasping of the female's genital region by the male's phallomere. The application of male accessory gland material to the genital region of females (Table 6) was ineffective as a stimulus to oöcyte development, which would tend to rule out possible chemical stimulation as a factor. The effect of mechanical stimulation during mating persists in *Diploptera* even after mating is completed.

ENGELMANN (1959) inserted glass 'spermatophores' into the bursa copulatrix of virgin *Diploptera* and found that 5 of the 8 females had begun to deposit yolk in their oöcytes 3–14 days after the operations (17–26 days after emergence). The results of this type of stimulation were not as clear cut as controls (mean length of the oöcytes in experimental females was only $0{\cdot}72 \pm 0{\cdot}04$ mm). He suggested that a glass spermatophore was 'too smooth to afford sufficient stimulation. Furthermore, normal mating may last up to several hours during which time the movements of the male provide a more efficient stimulation of the genital apparatus than that caused by deposition of a smooth artificial glass spermatophore.' We have already pointed out that the length of time a pair is *in copula* is not the important factor in stimulation. Insertion of the spermatophore into the bursa together with some type of movement of the male at about the time he releases the female after completing the act appear to be important in maximum stimulation of the female.

TABLE 6—EFFECT OF VARIOUS TYPES OF 'STIMULATION' OF THE GENITAL REGION ON SUBSEQUENT DEVELOPMENT OF THE OÖCYTES IN VIRGIN *Diploptera punctata*

Treatment	Age when treated (days)	Days after treatment	Oöcytes Mean ± S.E. (mm)	*N*
(1) Beeswax pellet inserted in bursa copulatrix	1 1	10 10	0·77 ± 0·04 1·30 ± 0·19	4 (−)* 3 (+)†
(2) Glass beads inserted in bursa copulatrix	1–2 1–2	8 8	0·72 ± 0·01 1·26 ± 0·16	19 (−) 5 (+)
(3) Dried spermatophores (expelled by mated females) moistened in Ringer's and inserted in the bursa copulatrix	1 1	11–12 11–12	0·69 ± 0·02 1·33 ± 0·12	9 (−) 5 (+)
(4) Beeswax plug inserted in uterine opening	1	10	0·68 ± 0·01	13 (−)
(5) Ovipositor cut off below the base	1 1	11 11	0·71 ± 0·01 1·63	11 (−) 1 (+)
(6) Ovipositor cut off at the base	<1 <1	7 7	0·72 ± 0·04 1·23	2 (−) 1 (+)
(7) Ovipositor cut off above the base	<1 2–4 2–4	8 11 11	0·69 ± 0·02 0·73 ± 0·02 1·14 ± 0·15	6 (−) 12 (−) 4 (+)
(8) All valves of the ovipositor pinched with forceps	<1	6–10	0·71 ± 0·02	9 (−)
(9) Fleshy tissue below base of ovipositor pinched with forceps	6 6	11 11	0·67 ± 0·004 0·86 ± 0·01	7 (−) 3 (+)
(10) Fine forceps rubbed between valves and at base of ovipositor	3 3 3	7 11 11	0·71 ± 0·03 0·72 ± 0·02 0·88 ± 0·01	3 (−) 3 (−) 2 (+)
(11) Hot needle applied to outer region of bursa	<1 <1	6 6	0·64 ± 0·03 1·16 ± 0·04	2 (−) 3 (+)
(12) Hot needle inserted into bursa	5	6	0·73 ± 0·03	5 (−)
(13) Hot needle applied to ovipositor	5	6	0·73 ± 0·03	6 (−)
(14) Warm needle applied to ovipositor	5 5	10 17	0·69 ± 0·02 0·69 ± 0·01	6 (−) 5 (−)
(15) Drop of hot water (about 96°C) applied to genital region	1 1	10 10	0·73 ± 0·02 0·92	4 (−) 1 (+)
(16) Male accessory glands (central lobes) crushed into a paste and applied to ovipositor	6 6	11 11	0·67 ± 0·02 0·96	7 (−) 1 (+)
(17) Viscous secretion of male accessory glands, taken from recently deposited spermatophores, applied to ovipositor	7 7	7 11	0·74 ± 0·03 0·73 ± 0·03	4 (−) 5 (−)

* (−) = No growth of oöcytes beyond that found in virgin females.
† (+) = Definite growth of oöcytes.

The effect on oöcyte development of various types of 'stimulation' of the genital region of virgin *Diploptera* is shown in Table 6. Positive and negative results of the various treatments are listed separately. It is evident that oöcyte development in some females can be induced by certain types of mechanical stimulation of the genital region. The most effective stimuli are those which involve inserting an object (beeswax, glass bead, old moistened spermatophores) into the bursa copulatrix, which may indicate that pressure on the bursa is involved in stimulation. ENGELMANN (1959) found that after removing the three pairs of gonapophyses from 12 virgin females, 10 deposited yolk in the oöcytes after a few days. He concluded that afferent nerves from the ovipositor probably were stimulated by the excision of the gonapophyses, which resulted in activation of the corpora allata and that the 'conclusion that sensory receptors on the gonapophyses receive the stimuli during the act of mating or parturition seems, therefore, justified'. However, 3 of his *Diploptera* lacking ovipositors eventually mated and formed oöthecae in the normal interval taken by mated females. He therefore concluded that sensory receptors on the gonapophyses and on other parts of the genital apparatus receive the stimuli resulting from mating. We have confirmed ENGELMANN's work regarding stimulation of the oöcytes resulting from cutting off the ovipositor; in addition, other types of stimulation were more or less effective in stimulating a small number of females (Table 6). However, these experiments simply show that various types of mechanical stimulation in the genital region may serve to induce oöcyte development in some females. It is not possible to localize the site of stimulation using the methods listed in Table 6 and these experiments do not pinpoint the site of the receptors that receive the mating stimuli.

We removed the ovipositors from last instar female larvae of *Diploptera*, and those that metamorphosed and mated were kept for 2 weeks. Thirty-five adult females were obtained from operated larvae. Of these, 13 completely lacking ovipositors mated (7 had spermatophores transferred normally and 6 lacked spermatophores or they were dropped by the males without inserting them in the bursa); 11 of the females oviposited within 12–14 days after mating, and the other 2 were dissected 10 and 14 days after mating and had oöcytes 1·18 and 1·58 mm long, respectively. Five females that had abnormally regenerated ovipositors (some of the valves were present but were abnormally shaped) oviposited within 2 weeks after mating. Nine females whose ovipositors appeared normal after regenerating also oviposited within 2 weeks after mating. Eight females that lacked ovipositors but did not mate had oöcytes ($0{\cdot}72 \pm 0{\cdot}03$ mm) typical of virgin females when dissected 10–14 days after emergence. It is evident that the gonapophyses are unnecessary for the female to receive stimulation during mating. That males can mate with females lacking ovipositors indicates that these structures are not needed for the male to clasp the female. However, since the spermatophores were not transferred properly to some of the operated females, it may be that the ovipositor aids in the proper orientation of the male during copulation so that the spermatophore can be inserted normally into the bursa. Cutting off the ovipositor may result in oöcyte development in some females, but it is possible that some other receptors in the region of the ovipositor were stimulated during the operation. A careful study is needed of the relationships of the external genitalia during copulation in *Diploptera*, as has been done in other species of cockroaches (CHOPARD, 1919; GUPTA, 1947; KHALIFA, 1950), before the ovipositor is definitely implicated in transmitting mating stimuli. At present the evidence indicates that the receptors for receiving mating stimuli in *Diploptera* are present in the genital region—perhaps near the bursa or near the base of the ovipositor, but not necessarily on the ovipositor itself.

Inhibition of oöcyte development during pregnancy

ENGELMANN (1959) removed the oöthecae from 13 females of *Diploptera* 0–41 days after onset of pregnancy. None of these females matured eggs within 31 days.

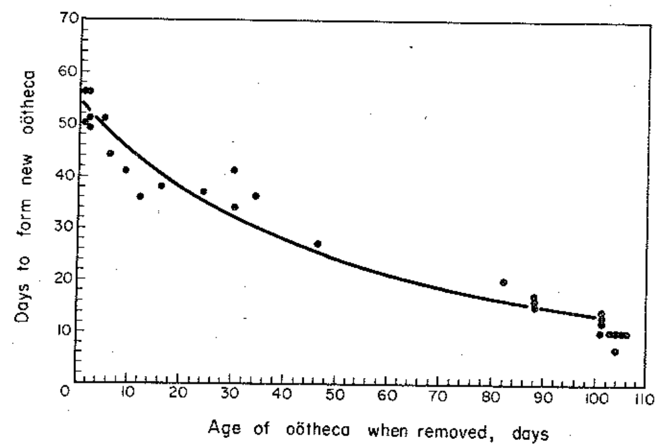

FIG. 2. Relationship between the age of the oötheca at the time it was removed from the uterus of *Diploptera* and the time required to ovulate again. Each point represents 1 female. The 5 females that produced a second oötheca in 10 days and the one that ovulated in 7 days had given birth naturally.

An additional 5 females that had their oöthecae removed 3–5 days after ovulation formed another oötheca 42–71 days later. ENGELMANN concluded that the period required for egg maturation after removal of the egg case was roughly comparable to that observed in virgins. Since egg maturation was inhibited throughout the period of pregnancy (75 days), he postulated an additional inhibitory action from the oötheca, and 'in analogy with the known situation in *Leucophaea*, it may be due to a humoral factor'.

We removed the oöthecae from 50 females at different stages of pregnancy. Twenty-three oviposited again within the period (64 days) of the experiment. As has been found in *Blattella* and *Pycnoscelus* (ROTH and STAY, 1961a, b), the younger the oötheca when removed from the uterus, the longer the time interval to form another egg case (Fig. 2). The closer to parturition when the oötheca was removed, the more rapid was the renewed growth of the oöcytes and this growth was more rapid than occurs in virgins (if it occurs); only 2 virgin females oviposited and 3 others had well-developed oöcytes within 64 days after emergence (Fig. 1). Of the 27 other females that had not ovulated, 9 had well-developed oöcytes ($1{\cdot}54 \pm 0{\cdot}03$ mm) 11–59 days after the oöthecae were removed; 3 of these 9 females whose oöthecae were 82 and 95 days old when removed had oöcytes which averaged 1·51 mm 11–13 days after removal. Eighteen females showed little or no oöcyte development ($0{\cdot}70 \pm 0{\cdot}02$ mm) 52–64 days after removal of their oöthecae; in 15 of these females the oöthecae were removed less than 1–12 days after onset of pregnancy and 3 had the egg cases removed 24 or 42 days after oviposition.

To determine whether inhibition of the oöcytes by the uterine eggs during pregnancy is due to nervous or humoral factors, 12 pregnant females (histories unknown) were taken from cultures and their nerve cords severed; in addition, 1 female had her cord severed 6 days after oviposition. The females were dissected at various periods after the operation, or they were kept until parturition occurred and their oöcytes were measured. The lengths of the uterine embryos of those females that were dissected prior to parturition were also determined. The results are shown in Fig. 3. For comparative purposes the relationship of oöcyte size to embryos of increasing length in normal females is given. Twelve of the 13 operated females showed a marked growth of the oöcytes. The 6 females that had their nerve cords severed 11–18 days before parturition had mature oöcytes ($1{\cdot}73 \pm 0{\cdot}4$ mm) when they gave birth, matured in spite of the presence of an oötheca in the uterus. It is evident that cutting the nerve cord in these pregnant females caused the corpora allata to become active, indicating that inhibition is due to a nervous stimulus as it is in *Blattella* and *Pycnoscelus* (ROTH and STAY, 1959, 1961a, b).

ENGELMANN (1960) severed the nerve cord of *Diploptera* females 2 days after ovulation and found that no oöcyte development occurred in 30 days. Eight pregnant females that had their cords severed 30–40 days after ovulation also showed no oöcyte development 8–24 days later. However, 12 of 14 animals operated on 50–75 days after ovulation began to mature their eggs within 9–27 days. He concluded that some nervous component is involved in inhibiting the corpora allata during gestation, but perhaps only during the last phase of pregnancy, and perhaps during late pregnancy the female is more sensitive and, therefore, more readily activated.

One of our females (numeral 60 attached to open circle in Fig. 3) whose cord was cut 6 days after oviposition had almost mature oöcytes when examined 60 days later; this is roughly the time one would expect for another oötheca to be formed if the first one is removed 6 days after ovulation (Fig. 2). From the size of the embryos it is possible to estimate the length of time the other 11 females were pregnant when their nerve cords were severed (based on unpublished measurements of known-aged embryos). The estimated length of time these females were pregnant when their cords were severed was as follows (the numbers in parentheses refer to those shown attached to open circles in Fig. 3, reading from left to right); (23) = 32 days; (19) = 50 days; (14) = 55 days; (19) = 50 days; (23) = 70 days. The 6 females that gave birth had their nerve cords severed about 85–92 days after oviposition (based on a mean gestation period of 103 days). These results indicate that nervous inhibition operates over a wide period in pregnancy, actually from at least

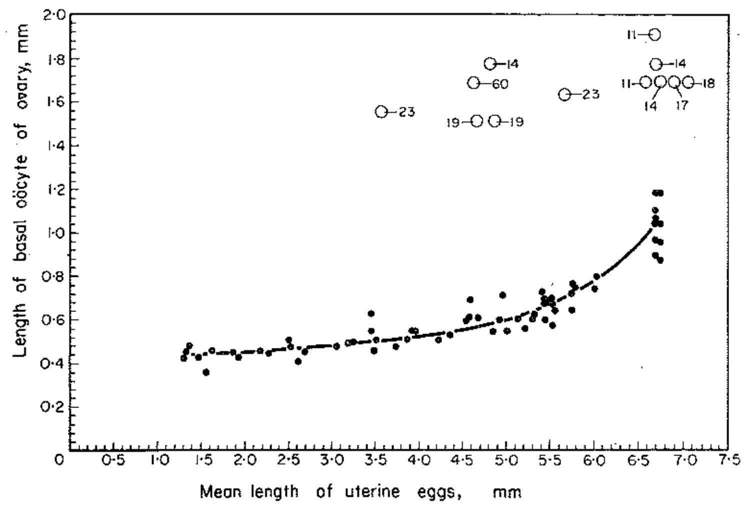

FIG. 3. Relationship between the sizes (mm) of uterine eggs and basal oöcytes in the ovary, and the effect of nerve cord severance on oöcyte development in pregnant females of *Diploptera punctata*. Solid circles = normal females. Each plotted point represents measurements of 1 female. The uterine-egg measurements are mean lengths of all the eggs in each oötheca; the oöcyte measurements are based on one oöcyte from the same female. The histories of the females were unknown. However, the smallest uterine egg measurements represent recently formed oöthecae. All values beyond 6·5 mm were from females that were ready to give birth or had just given birth. Open circles = oöcytes and uterine-eggs (except for those females that had given birth) of females with severed nerve cords. The numerals attached to these circles indicate the number of days the nerve cord had been cut prior to measuring the oöcytes. Eleven of the females were of unknown histories; 1 female had her cord severed 6 days after oviposition and was dissected 60 days later (circle with number 60 attached). The lengths of uterine eggs of nerve-cord-severed females that gave birth were not measured but are plotted at 6·5–6·8 mm. Hatching eggs reach a length of about 6·9 mm (ROTH and WILLIS, 1955).

as early as 6 days after ovulation. It is probable that had ENGELMANN waited a longer period than 30 days after nerve severance of females in the early stages of pregnancy, the oöcytes would have matured prior to the normal time required for gestation (about 75 days under his conditions).

ENGELMANN (1959) found that the corpora allata become active shortly before parturition and reach a peak of activity on the second or third day after parturition. Consequently, yolk deposition in the oöcytes begins on the second or third day before birth, and at parturition the basal oöcytes are about the size of those of a 3-day-old mated female; thus the time required for the oöcytes to mature, following parturition, is about 3 days less than for the first ovulation following mating (Fig. 1). ENGELMANN suggests that the deposition of yolk in the oöcytes prior to parturition is due to the rhythmical contraction of the abdomen during the later days of pregnancy. 'The resulting movements of the egg case in the genital apparatus presumably initiate the activity of the corpora allata before parturition.'

By sealing the genital segments with cement 2–8 days prior to parturition (estimation based on a mean gestation period of 103 days), we prevented 15 females from giving birth. About 5–7 days after birth theoretically should have occurred, the females were sacrificed and their oöcytes were measured. In most of the individuals the embryos in the uterus were dead at termination of the experiment. The results are shown in Table 7. Two females had oöcytes smaller than those found at parturition, and 3 females had oöcytes about the size of those at parturition. However, the other 10 all had oöcytes that were as large as those found in females several days after parturition (cf. Table 1). Since parturition may occur 99–110 days after oviposition, it was unknown when these females would normally have given birth, but it is apparent that the oöcytes had continued to grow in 10 females even though parturition was prevented.

TABLE 7—EFFECT OF PREVENTING PARTURITION ON DEVELOPMENT OF THE OÖCYTES IN *Diploptera punctata*

Days pregnant when genital segments were sealed	Days pregnant when oöcytes were measured	Oöcytes (mm)
95	110	0·67
95	110	1·51 (+)*
95	110	1·51 (+)
96	111	1·51 (+)
96	111	1·65 (+)
97	108	0·72
99	110	1·51 (+)
100	111	1·66 (+)
100	108	1·51 (+)
100	108	1·38 (+)
100	108	1·61 (+)
101	108	0·67
101	108	0·81
101	108	0·96
101	108	1·29 (+)

* (+) = Oöcytes larger than those normally present at parturition.

In attempting to prove that parturition serves as a stimulus to oöcyte development, ENGELMANN (1960) removed the egg cases from females 2–10 days before parturition should have occurred. Although he avoided stimulating the genital apparatus during the operation, 12 of 16 animals matured their oöcytes in 10–21 days. He accounted for these 12 cases as probably being due to maturation having already been initiated at the time of removal of the egg cases; and he suggested that stimulation of the genital apparatus prior to parturition by the movement of the egg case may suffice to induce complete egg maturation. ENGELMANN also stated that this experiment confirmed his previous observations 'that parturition as such is a stimulus to the corpora allata since these females did not respond immediately after gentle removal of the egg case'.

Our experiments on severing the nerve cord of females do not support ENGELMANN's hypothesis. If the cord is severed 11–18 days prior to parturition, the oöcytes may mature by the time the female gives birth (Fig. 3). This indicates that the inhibition of the oöcytes during pregnancy is under nervous control at this time. It also is evidence against the hypothesis that movement of the egg case prior to parturition, and also that parturition itself, stimulates the corpora allata; a severed nerve cord would prevent the transmission of such stimuli to the brain.

The fact that the corpora allata did not respond 'immediately' after gentle removal of the egg case in ENGELMANN's experiments is not evidence that parturition stimulates the corpora allata. The rapidity with which the corpora allata respond (as measured by oöcyte development or maturation) to removal of the oötheca varies with the stage of pregnancy at the time of removal of the egg case. This inverse relationship between the time required for a second ovulation and the age of the first oötheca when removed from the uterus (Fig. 2) may be due in part to the fact that the oöcytes are smaller during the early days of pregnancy (Fig. 1) and therefore take longer to mature. ENGELMANN (1959) found that when the oötheca was removed at about the middle of the gestation period and the female was mated within 4–6 days later, the oöcytes matured in about 9 days; when the oötheca was removed and the female was not subsequently mated, ovulation occurred in about 41 days. Removing the oötheca obviously differs in its effect from mating, and one may interpret this to mean that in the former operation one is simply removing an inhibitory factor whereas in the latter the corpora allata are being stimulated to greater activity. As ENGELMANN suggested, the corpora allata apparently respond more readily in the later stages of pregnancy when inhibition (by the oötheca) is removed. Since inhibition during most of the gestation period is due to nervous stimuli, and mechano- or pressure-receptors are probably involved in transmitting stimuli from the stretched uterus (as the embryos increase tremendously in size; ROTH and WILLIS, 1955, 1958), it is conceivable that the receptors eventually become adapted during late pregnancy. Or adaptation occurs in the central nervous system rather than in the sense organs (V. G. DETHIER, personal communication). Several days before parturition, adaptation may reach a state where inhibition of the corpora allata is markedly reduced, the glands begin to secrete, and the oöcytes mature. Activity of the corpora allata continues or increases when the inhibition is completely removed as a result of birth of the young. Birth does not necessarily afford additional stimulation, for the oöcytes continue to develop even though parturition is prevented experimentally.

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REFERENCES

CHOPARD L. (1919) L'accouplement et le fonctionnement de l'armure copulatrice des Blattidae (Orthoptères). *Bull. Soc. zool. Fr.* **44**, 347–353.

ENGELMANN F. (1959) The control of reproduction in *Diploptera punctata* (Blattaria). *Biol. Bull., Woods Hole* **116**, 406–419.

ENGELMANN F. (1960) Mechanisms controlling reproduction in two viviparous cockroaches (Blattaria). *Ann. N.Y. Acad. Sci.* **89**, 516–536.

GUPTA P. D. (1947) On copulation and insemination in the cockroach *Periplaneta americana* (Linn.). *Proc. nat. Inst. Sci. India* **13**, 65–71.

HAGAN H. R. (1941) The general morphology of the female reproductive system of a viviparous cockroach, *Diploptera dytiscoides* (Serville). *Psyche, Camb. Mass.* **48**, 1–9.

KHALIFA A. (1950) Spermatophore production in *Blattella germanica* L. (Orthoptera: Blattidae). *Proc. R. ent. Soc. Lond.* (A) **25**, 53–61.

ROTH L. M. and STAY B. (1959) Control of oöcyte development in cockroaches. *Science* **130**, 271–272.

ROTH L. M. and STAY B. (1961a) Oöcyte development in *Blattella germanica* (Linn.) and *Blattella vaga* Hebard (Blattaria). (In preparation.)

ROTH L. M. and STAY B. (1961b) Oöcyte development in false ovoviviparous cockroaches. (In preparation.)

ROTH L. M. and WILLIS E. R. (1954) The reproduction of cockroaches. *Smithson. misc. Coll.* **122**, 1–49.

ROTH L. M. and WILLIS E. R. (1955) Intra-uterine nutrition of the 'beetle-roach' *Diploptera dytiscoides* (Serv.) during embryogenesis with notes on its biology in the laboratory (Blattaria: Diplopteridae). *Psyche, Camb. Mass.* **62**, 55–68.

ROTH L. M. and WILLIS E. R. (1958) An analysis of oviparity and viviparity in the Blattaria. *Trans. Amer. ent. Soc.* **83**, 221–238.

STAY B. and ROTH L. M. (1958) The reproductive behavior of *Diploptera punctata* (Blattaria: Diplopteridae). *Proc.* 10*th int. Congr. Ent.* **2**, 547–552 (1956).